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In Vitro Assessment of Botanicals Rich in Phytochemicals for Egg Hatchability Management *of Meloidogyne Incognita*

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Abstract

The current study was aimed at determining the nematicidal efficacy of Helianthus annuus and Chromolaena odorata in the management of M. incognita. The qualitative and quantitative screening of the phytochemicals present in the extracts of tested botanical was done with the gas chromatography flame ionization detector and spectrophotometric analysis where flavonoids, alkaloids, saponins, phenols, tannins, steroids and glycosides were obtained at varying concentrations. The botanical extract significantly enhanced the inhibition of juveniles hatching individually when compared to the control (P<0.05). The maximum percentage egg hatch inhibition recorded in tested botanicals at 72 hours of exposure in 100% concentration were Helianthus annuus = 68% and Chromolaena odorata = 89.33%. The extract from Chromolaena odorata was more infective due to the presence of terpenoids which are not found in the extract of Helianthus annuus. The study thus, suggests that the identification, isolation and bio-assaying of phytochemicals in the tested botanicals could lead to nematicidal discovery as a good alternative for the management of M. incognita for an eco-friendly and sustainable agriculture.

1. Introduction

The effective management of pest and pathogens of plants requires multiple alternative strategies which must be very effective, eco-friendly, cheap, readily available to meet up the food safety and sustainability in the present environmentally conscious world as to meet up the global standard (Ebuete, Ebuete & Berezi, 2022). Several strategies such as cultural practice and biological control are been advocated. The cultural practice in most cases could not produce the desired result because of the multi-dimensional mechanisms for survival deployed by plant pathogens, thus, the bio-control strategies is currently accepted as the most preferred. Biological control is the use of living organism or the product from the organism to control or eliminate the economic losses to crop plants caused by pest in the agricultural sector (Sidhu, Kumar & Madhu, 2017). It also involves the use of genes and phytochemicals from living organisms to control the damage caused by Pathogens to plants (Contrell *et al,* 2012). Biological control is a more stable longer lasting, cheap and environmental friendly approach to pest management (Cook, 1987). Currently there is an increase in research on the use of natural products from plants with focus on identification of phytochemicals of natural origin for the control of plant parasitic nematode. Plants are chemical factories that easily transform relatively simple substances such as water and air into organic substances such as protein, enzymes, oils and secondary metabolites that are poisonous to several pathogens of plant (Bebber *et.al.* 2014). Some of these substance releases by plant have also been reported to play several roles in granting protection and defense to plant against their invaders (Sidhu, Kumar & Madhu, 2017). Root knot nematode (*Meloidogyne species*) is a major constrain to crop plant production globally, it is identified as one of the plant parasitic nematode of economic importance (Ames *et.al.* 1997; Jones, *et. al.*, 2013; Bebber, *et.al.* 2014; Sidhu *et.al.* 2017; Karuri, *et.al.2017*; Harjat *et al,* 2017; Abdusalam

et.al. 2017; Abraham *et.al.* 2018; Chrisostomos *et al,* 2018; Patil, 2020; Iliya *et al,* 2021; Moh *et al,* 2022). The most damaging species to crops are *M. javanica, M. hapla, M. arenaria* and *M. incognita.* Root knot nematodes are found in all tropical and subtropical regions of the world (Trudgill and Blok, 2001). It is also reported that root knot nematode cause loss ranging from 10% to 69% in the agricultural sector (Olowe, 2009).

Chemical control which happens to be the most effective of plant parasitic nematode control strategies has been several criticized to create potential hazard to the environment and health of human beings, also, there has been deregistration of some of these hazardous nematicides leading to scarcity and high cost of the available chemical nematicides. This have caused and increasing pressure on nematologies and farmers to source for non-chemical nematicides that are eco-friendly to satisfy the concept of organic agriculture (Adekunle & Fawole, 2003). This therefore create a shift to the deployment of strategies that are eco-friendly such as the use of natural resources, with nematicidal efficacies from plants like root exudates, biochers, amendments and extracts to control plant parasitic nematodes (Chitwood, 2002). The major phyto-nematode control research at present is the study of herbal preparations rich in allelochemicals with nematicidal activities that are eco-friendly and biodegradable. Several plant species have been tested to identify the source of nematicidal substances and alot of them have shown promising results for the control of plant parasitic nematodes (Adegbite & Adesiyan, 2005). Aqueous and organic extracts from parts of several plants such as neam, bitter leaf, moringa, siam, African basil, fluted pumpkin and among others have been reported to contain nematicidal properties that are inhibitory to egg hatching and development of root knot nematodes (Ajayi, 1990; Adegbite & Adesiyan, 2005; Abraham et.al. 2018). Several of researchers have also reported that the nematicidal potency in plant is linked to the presence of constituent phytochemicals such as alkaloid, phenols, flavonoids, saponins, steroid, tannins, glycosides, Terpenoids and fatty acids (Chitwood, 2002; Adekunle & Fawole, 2003). The uses of plant extracts have the advantages of being effective, cheap, safe and readily available as a good nematode control strategy (Mangala & Mauria, 2006). These therefore spur us to investigate nematicidal efficacy of the extracts of *Helianthus annuus* and *Chromoleana odorata* on egg hatchability of *M. incognita* in vitro.

2. Materials And Methods

Matured leaves of sunflower (*Helianthus annuus*) and Siam (*Chromoleana odorota*), collected from the demonstration farm of the department of Crop and Soil Science, Faculty of Agriculture, University of Port-Harcourt River State as shown in (Table 1).

Table 1: Information about the two tested botanical used in this study

English name	Botanical name	Plant part used
Sunflower	*Helianthus annuus*	Leaves
Siem	*Chromoleana odorata*	Leaves

Source: Researcher, 2022.

2.1 Preparation of Plant Extracts

Leaves of tested botanicals plucked from the weeds were thoroughly washed and air dried on the benches of the laboratory at room temperature for 15 days. The dried leaves were grind to fine powder using an electrons blender. 100 g of each of the ground materials were introduces into separate 500 ml flat bottom flash containing 400 ml of 95% ethanol and shaken on a rotary shaker at 120 rpm for 24 hours. The content was carefully filtered using the Whatmann No1 filter paper, the filtrate was vacuumed using a rotary evaporator at 40^{o}c to get organic crude extract (stock solution). The extracts were prepared into 10%, 25%, 50% and 100% concentration with distilled water dilution.

2.2 Extraction and Standardization of Nematode Eggs Inoculum

Heavily galled roots of okra were properly washed with running water to remove soil particles and other debris. The root containing egg masses were chopped into small pieces and introduced in 500 ml flat bottom flash containing 200 ml of 0.5% sodium hypochlorite solution and shaken vigorously for 4 minutes as to digest the gelatinous matric covering the eggs. The content was poured through a 200-mesh (75 µm) placed over a 500-mesh (25 µm) into a container. The eggs trapped in the 500-mesh sieve were washed with slow running water into the container in order to remove the NaOcl. The roots trapped by the 200 mesh sieve were washed twice with water into a container to obtain more eggs. The collected eggs were topped with water to 100 ml. 1 ml of the suspension was pipette after bubbling air out and mounted on a stereomicroscope and counted. The counting was done thrice to obtain the mean number of egg in 1 ml of suspension.

2.3 In Vitro Hatchability Test

3 ml of egg suspension prepared (± 150 eggs/3 ml) were introduced into different concentrations of tested botanical extract of 10%, 25%, 50% and 100% in different petri dishes. The preparation was done in triplicates and the petri dishes with distilled water serve as control. The number of egg hatched was counted under binocular microscope after exposing the test to 24, 48 and 72 hours.

2.4 Gas Chromatography-Mass Spectrometry (Gc-Ms) Analysis

The gas chromatography is a 30 m column composed of 0.25 mm id, 0.25 µm thickness film, HP-SMS (5% biphenyl) dimenthylpolysiloeane capillary column with the following conditions: 240°c injector temperature, a column temperature, 2 minute isothermal at 50°c which is programmed to 280°c at 6°c in every minute that hold for 2 minutes, ion source temperature, 200°c detector temperature, (300°c). Helium gas carrier at the rate of 1ml/minute. Spectra were collected in the El mode with 70eV ionization energy when the effluent of the Gas Chromatography column was introduced directly into the source of ion of the mass spectrometry. The sector mass analyzer was programmed to scan from 40-400 *amu* in 5 seconds. These data were collected from the Department of Pharmacognosy and Phytotherapy, Faculty of Pharmaceutical Sciences, University of Port-Harcourt; Rivers State.

2.5 Data Analysis

Data on egg hatch inhibition were analyzed using the square root transformation $(x + 0.5)^{1/2}$ where x represent the number of J_2 and 0.5 is constant (Gomez and Gomez, 1984) after the number of J_2 hatched have been normalized. The transformed values where further analysis using the analysis of variance (ANOVA) procedures following Gomez and Gomez (1984); the least significant difference (CSD) at 5% was used in comparing mean treatments.

3. Results And Discussion

Table 2. Effect of extracts of *Helianthus annuus* and *Chromolaena odorata* on juvenile hatching at 10%, 25%, 50% and 100% concentrations at 24, 48 and 72 hours of exposure in vitro.

Botanical	Exposure Time	10%	% inhibition	25%	% inhibition	50%	% inhibition	100%	% inhibition
Helianthus	24	118	20.89	109	27.33	100	33.33	76	48.89
Annuus	48	05	29.33	99	33.78	75	50	69	54
	72	95	34.66	92	38.66	69	53.56	48	68
Chromolaena	24	63	58	59	60	55	63	43	71
Odorata	48	40	73.33	35	76.66	21	86	19	87
	72	41	75.66	32	78.66	19	87.33	16	89

Sources: Researcher, 2022.

Each value is expressed as mean of three replicates results, shows that all the extracts of the tested botanicals were effective in inhibiting the egg hatching of *M. incognita*. There is also a significant difference in the percentage egg hatching in all the treatments. The maximum percentage egg hatching of 68% and 89% were recorded at 72 hours of exposure in the extracts of *H. annuus* and *C. odorata* respectively.

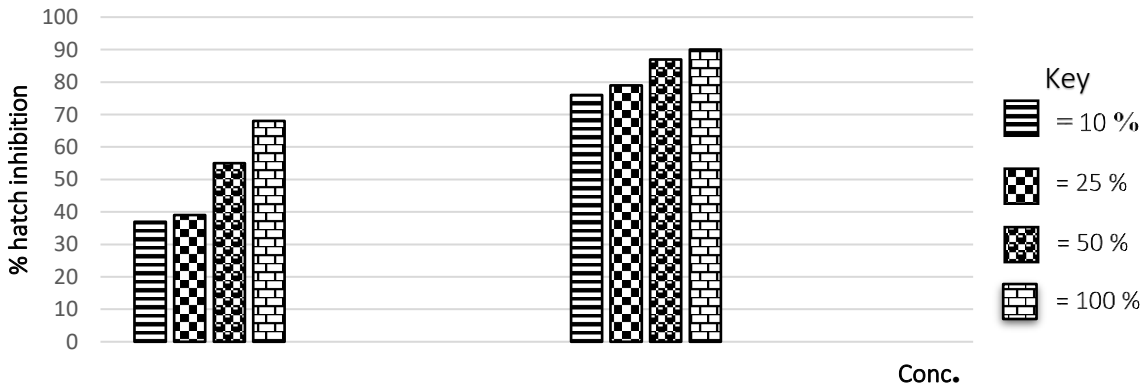

Figure 1: Effect of different concentrations (10%, 25%, 50% and 100%) on percentage hatch inhibition at 72 hours of exposure

The results shows that the extract of the botanicals at different concentration were able to inhibit egg hatching at different concentration. The extract of *C. odorata* was more efficious even at the lowest concentration of 10% at 75.66% egg hatch inhibition, and the efficacy of egg hatch inhibition is concentration dependence. The maximum percentage egg hatch inhibition at 72 hours at exposure of *H. annuus* and *C. odorata* 76% and 89% respectively.

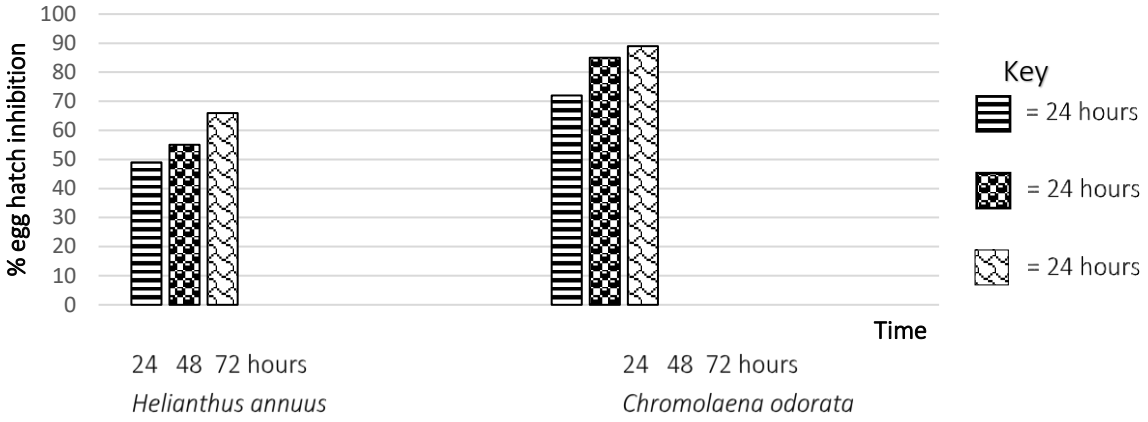

Figure 2: Effect of time of exposure (24, 48 and 72 hours) on the percentage egg hatch inhibition of 100% treatment at botanical extracts

Result shows that, as the time of exposing of treatments to eggs of *M. incognita* increases, the inhibitory effects of egg hatching also increase. This explains that there is a strong relationship between concentration and period of exposure of treatment on egg hatching of *M. incognita.*

Table 3: The qualitative and quantitative analysis of the phytochemicals present in the botanical extract of *H. annuus* and *C. odorata* in mg/g.

	Qualitative	Analysis	Quantitative	Analysis
Phytochemicals	H	C	H	C
Alkaloids	+	++	6.32	11.9
Flavonoid	+++	+	84.24	7.8
Saponins	++	+++	6.92	322.8
Tannins	++	++	10.95	14.4
Steriods	-	-	0.00	0.00
Phenol	+++	++	309.86	37.9
Terpenoid	-	++	0.00	38.7

Source: Researcher, 2022.

Results of the quantitative and qualitative analysis of the phytochemical present in the extract of *H. annuus* and *C. odorata* in mg/g, +++ = abundantly present ++ = moderately present, + = traceable and - = not detected. It shows that the extracts of the tested botanicals are rich in several phytochemicals such as alkaloids, flavonoids, saponins, tannins, phenols and terpenoids in different composition of the extracts, which may have resulted to the high efficacy of the tested botanicals in egg hatch inhibition of *M. incognita* in vitro.

The emphasis on the use of extracts from plants for the control of plant parasitic nematodes is becoming appealing because of the compounded problems generated by the chemical nematicides (Kerry, 1990). The use of plant extracts as nematicides have the advantages of being readily available cheap, easily degradable and eco-friendly (Fawole, 2009). Botanicals, being environmentally-safe in an environmentally conscious world also hold promise to their acceptance and use by poor farmers. Several botanical species have been tested to identify the source of nematicidal substances and many of them have shown promising results for the control of plant parasitic nematodes (Adegbite & Adesiyan, 2005). The in vitro studies of the extracts of *Helianthus annuus* and *Chromolaena odorata* were very effective in inhibiting egg hatching of *M. incognita*, although the nematotoxic potency varies among the two tested botanicals in all the concentration even at the lowest concentration of 10% as the effect can easily be recognized when compared to the control.

The percentage egg-hatches inhibition of *M. incognita* was concentration and exposure of time dependant, (i.e) the higher the concentration and time of exposure, the higher the percentage egg hatch inhibition (few

juveniles hatching). This agrees with earlier reports of Adegbite and Adesiyan, (2005) that the root extracts of *C. odorota, J. curcas, A. indica* and *R. communis* cause egg hatch inhibitory at different levels, and the effect increased as the concentration of treatments and exposure time increases. Furthermore, the aqueous extracts of *V. amygdatina, A. indica, M. oleifera* and *O. gratissimum* exhibited different degrees of nematotoxicity of *M. incognita* and the efficacy was due to the direct contract of the extracts with the egg ensured that the active ingredients in the leaf extracts were effectively delivered to the eggs.

The phytochemical screening of the extracts of *H. annuus* and *Chromolaena odorata* also showed that the tested botanicals have abundance of chemicals of nematicidal importance such as alkaloids, flavonoids, saponins, steroids, phenols, terpenoids and glycosides which may have directly or indirectly caused the inhibitory effects on egg hatching of *M. incognita.* This agrees with the initial findings of Adegbite (2003) and Fawole (2009), reported that the phytochemicals in botanicals inhibits egg hatching by either affecting the embryonic development or killed the egg or even dissolved the egg masses. It also appeared that the egg hatching inhibitory effect is due to the permeability of egg shall to the toxic material contained in the botanical extracts used in the investigation thereby, killing the developing juveniles in the egg. This then agrees with the finding of Adegbite and Adesiyan (2005), that the egg hatch inhibition of *M. incognita* might be due to chemicals present in the extracts that possess ovicidal or larvicidal properties which either affected the embryonic development or kill egg directly, they also reported that, the efficacy of plant extract on egg hatch inhibition is linked to the phytochemicals present in the extract as bioactive substances. Tsai (2000), have reported that phytochemicals produced in plants may control nematodes by direct killing, preventing penetration by causing paralyses, causing the loss of host-finding ability, repulsion or by an unknown mechanism. Abdulsalam *et.al.* (2017), also reported that the nematicidal effect of crude extracts may probably be due to higher content of certain oxygenated compounds which are considered to possess lipophilic properties that allow them to dissolve the cytoplasmic membranes of nematode cells and their functional groups interfering with enzyme protein structure.

The egg hatch inhibitive effect of *M. incognita* has been linked to the present of phytochemicals present in the extracts of botanical that might have led to several mechanisms that have resulted to the efficacy. This agrees with the finding of Abdulsalam *et. al.* (2017) who reported that, the mode of action of phytochemicals on plant parasitic nematodes may include inducer of resistance, antifeedant, repellent, deterrent, growth disruption, J2 toxicant and ovicidal properties. This also corroborated with the finding of Stirling (2001), who also reported that, the inability of nematode to detect oxidants produced in roots is due to presence of phenols in the plants. He also reported that the oxidation process which catalysed the production of reactive oxygen species (ROS) in the cell well that result to plant defence is induced by phenolic compounds. Fatoki and Fawole, (2000); Mashela and Ngobeni (2015) reported that alkaloids are very toxic to insects and plant parasitic nematodes. Thoden *et al,* (2009), stated that the mode of action of alkaloids was protease inhibition. Tannins was reported by Fatoki and Fawole (2000), to have acted as defence mechanism in plants against several pathogens. Kumbasli *et al,* (2011), also reported that tannins induced morphological changes on pathogens by acting on cell membranes to destabilize cytoplasmic and plasma membranes, inhibits extracellular microbial enzymes and metabolic substances required for microbial growth. Ibrahim and Strous (2013) reported that saponins induced the formation of transmembrance pores by causing reduction in membrane integrity of cells. They also revealed that the nematicidal activity of saponins could be attributed to their ability to inhibit cholesterol accumulation in egg and larva.

The nematicidal efficacy of *Chromolaena odorata* have been reported by several workers such as Tsai, (2000), Adekunle and Fawole, (2003), Thoden *et al,* (2009), Karuri *et.al.*, (2017). Several works have also reported on antimicrobial activity of Helianthus annuus and its use as herbal remedy for several diseases and infection. Amakura *et al,* (2013), reported on the phytochemical constituent and characteristic of antioxidant marker from *H. annuus* seed extract; Divivedi *et al,* (2015), also evaluated the leave extracts of *H. annuus* for the antidiarrheal and antihistaminic activity; Jaramillo *et al,* (2016), also reported on the antifungal and antibacterial efficacy of extracts from parts of H. annuus. The percentage egg hatching inhibition at all concentration was significantly different and extract of *Chromolaena odorata* displayed a maximum percentage egg hatch inhibition of 98% at 100% concentration at 72 hours of exposure and *Helianthus annuus* had 68% of maximum percentage egg hatch inhibition. The extract of *C. odorata* was more effective than that of *H. annuus.* This is due to the composition of saponni, alkaloids (322.8 and 11.9 mg/g respectively), and the present of terpenoid *C. odorata* which is absent in the extract of *H. annuus.* This finding corroborated with the finding of Jones *et.al.*(2013), who reported that effects of botanical extracts from different species at the same concentration and period of exposure could therefore have different effects since response to allelochemical is composition specific.

4. Conclusion

The finding of the present studies have provided tangible evidence to show that botanical phytonemaicides has bioactivity on *M. incognita* egg hatching inhibition. The suppressive inhibitory effect in egg hatching of *M. incognita* suggested that inhibition of egg hatching was one of the mechanism involved in the suppression of the population densities of plant parasitic nematodes since the egg stage is an important stage in the life cycle of nematodes that develop to juveniles which are the infective stage.

5. Recommendation

This study therefore recommends that.

1. Biological control in plant-parasite nematodes should be encouraged using Botanical Phytonemaicides

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